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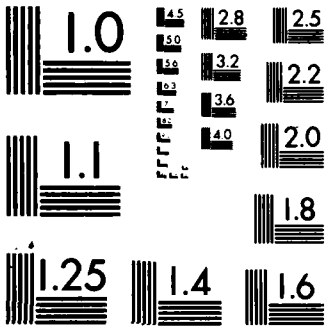

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EFFECTS OF LIQUID STORAGE IN A HIGH pH ANTICOAGULANT ON THE CIRCULATION OF BABOON PLATELETS

by

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Baboon platelet-rich plasma (PRP) was stored in a 150 ml filter flask at 22 ± 2 C for up to 15 days. The pH of the PRP was maintained at 7.4 by adding 6 N KOH or 6 N HCL during the storage period.
Reduced ^{51}Chromium platelet survivals immediately after transfusion and reduced lifespan values were observed when the platelet-rich plasma was stored at 22 C at pH 7.4 for 3 days or longer. Significant contamination was seen in the platelet-rich plasma after 7 days of storage.

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INTRODUCTION

Platelet concentrates stored at 22 C for up to 72 hours in polyvinyl-chloride (PVC, PL-146) plastic bags containing DEHP undergo a progressive loss of platelet function, as measured in vitro by platelet aggregation,[1] platelet dense body measurements and thrombin-induced release of ^{14}C-serotonin.[2] Recent data have shown that fresh platelets and platelet concentrates stored at 22 C for 3 days in PL-146 plastic bags show similar aggregation and secretion patterns in response to a combination of aggregating agents.[3]

Platelets stored at 22 ± 2 C with agitation in polyolefin plastic bags (PL-732, Fenwal Laboratories)[4] or in non-DEHP TEHTM (tri(2-ethylhexyl) trimellitate) PVC plastic bags (Cutter Laboratories)[5] have shown good maintenance of in vitro function and platelet posttransfusion survival. These containers permit better transport of oxygen into the platelets and removal of CO_2 from the platelets.

White and his colleagues have reported maintenance of in vitro platelet function and morphologic integrity of platelets stored in 150 ml filter flasks during storage at 22 ± 2 C for 15 to 21 days in a pH 7.4 CPD anticoagulant.[6] We report here on the in vivo circulation of baboon platelets isolated from blood collected in citrate-phosphate-dextrose (CPD), pH 9.0, and then stored as platelet-rich plasma at pH 7.4.

MATERIALS AND METHODS

From each of 7 baboons (Papio papio or Papio anubis) 200 ml of blood were collected in CPD, pH 5.5. In these studies the isolated platelet concentrates were labeled with ^{51}Cr, stored at 22 ± 2 C for as long as 4 hours, and reinfused.[7]

Each 200 ml volume of blood collected from 8 other baboons was stored in a CPD anticoagulant, pH 9, at a ratio of 8.6 parts blood to 1.4 parts CPD anticoagulant. The pH of the CPD anticoagulant was adjusted to 9.0 by the slow addition of 6 N potassium hydroxide (KOH) and sterilized by filtration through a 0.2 micron filter. The anti-coagulated blood was centrifuged at 200 X g for 20 minutes, the platelet-rich plasma was expressed into a 300 ml PVC transfer pack, and the packed red blood cells were returned to the baboon. The PRP was transferred aseptically into a 150 ml filter flask (Falcon #7103, Oxnard, CA), and the flask was placed in a controlled 22 ± 2 C incubator and stored for 3 to 15 days without agitation.

Prior to storage, and on the first, second, third, seventh and fifteenth days of storage, measurements were made of the pH at 22 C, platelet count per mm^3 by phase microscopy, bacterial culture on blood agar plates and in peptone broth, and mean platelet volume measurement using the H4 Channelyzer (Coulter Electronics, Hialeah, FL). In addition, the mean platelet volume was measured on a PRP sample fixed in 1% glutaraldehyde in a phosphate-buffered saline solution. The pH of the stored PRP was measured daily except weekends, and whenever it fell below 7.3 it was

raised to 7.4 by the drop-wise addition of 6 N KOH with a tuberculin syringe. Any spontaneous increase in pH above 7.6 was adjusted downward to 7.4 by the drop-wise addition of 6 N hydrochloric acid (HCL).

Platelet Transfusions and Survival Measurements

Fifteen baboons were studied: 14 each received 1 transfusion, and 1 baboon was given 2 transfusions. Seven of the transfusions were fresh platelet concentrates (PC) collected in standard CPD, pH 5.5; 2 were fresh platelet-rich plasma (PRP) collected in CPD, pH 9.0; 2 were CPD PRP stored at pH 7.4 for 3 days; 3 were CPD-stored PRP at pH 7.4 for 7 days; and 2 were CPD-stored PRP at pH 7.4 for 15 days.

^{51}Cr survival measurements were performed on the fresh and stored platelet concentrates by a modification of the method previously reported by Vecchione and associates.[8] The platelet-associated radioactivity in the PRP could not be determined using the previously described method; the ammonium oxalate washing of the ^{51}Cr-labeled-stored platelet-rich plasma produced a significant loss of platelet-associated radioactivity. Therefore, platelet-associated ^{51}Cr radioactivity was determined by isolating as many platelets as possible from 1 ml of labeled platelet concentrate by dilution with saline and centrifugation at 260 X g for 5 minutes. The platelets isolated in the supernatant were concentrated by centrifugation at 7000 X g for 5 minutes to remove free ^{51}Cr, and the platelet pellet was resuspended in 3 ml of saline and counted for radioactivity. The measured radioactivity was corrected for the platelets lost during the isolation procedure to determine the platelet-associated radioactivity in 1 ml of injectate.

Blood samples were obtained prior to and 30 and 60 minutes after transfusion, and then daily for up to 7 days as previously described.[8]

Blood volume was determined from the plasma volume measured with ^{125}I-labeled human albumin and the total body hematocrit.[9] Total body hematocrit was calculated by multiplying the peripheral venous hematocrit by 0.87.[10]

RESULTS

Three of the 9 platelet units became contaminated with bacteria after the seventh day of storage. One of the 3 contaminated units was transfused; this unit had been stored at 22 ± 2 C for 7 days and had the best survival of the 3.

The pH of the PRP was about 7.4 immediately after isolation and usually remained at this level for the first 3 days of storage (Table 1). By the seventh day of storage, the pH was about 7.0 and had to be adjusted to 7.4 with 6 N KOH. After 14 days of storage it was not necessary to adjust the pH of the PRP.

The platelet counts in the PRP stored at 22 C for 15 days are reported in Table 2.

The mean platelet volume of the platelets during storage at 22 C for 15 days is reported in Table 3. The effect of fixation of fresh and stored platelets in 1% glutaraldehyde in phosphate-buffered saline on the mean platelet volume is also reported in Table 3.

In limited studies, the ^{51}Cr platelet survival values for fresh baboon platelets isolated from whole blood were similar whether the high pH CPD anticoagulant in 2 studies or the normal pH CPD anticoagulant in 7 studies was used (Figures 1 and 2). The longer the period of platelet storage, the lower was the immediate posttransfusion survival value and the more rapid the removal of transfused platelets from the circulation (Figures 1 and 2).

DISCUSSION

The method of storing PRP in plastic flasks and making daily adjustments of the pH to 7.4 was time-consuming and cumbersome, requiring frequent entries into the container and resulting in a 33% bacterial contamination rate. There was a progressive loss of platelets during storage at 22 ± 2 C for 15 days, a finding similar to that reported by White and his colleagues.[6] The data were inadequate to assess the changes of the mean platelet volume during storage at 22 C and the effect of platelet fixation in 1% glutaraldehyde in phosphate-buffered saline on mean platelet volume.

White reported good maintenance of platelet morphology and in vitro function during storage of PRP for 15 to 21 days at 22 ± 2 C at pH 7.4.[6] Even though we followed this outline in our study, in vivo circulation was diminished and the lifespan of the platelets was reduced.

During platelet storage at 22 ± 2 C in the CPD anticoagulant at a high pH, we observed a loss of platelets in vitro and significantly reduced immediate posttransfusion survival and lifespan values.

Our data indicate that this method to store platelets was not practical and the results were not acceptable.

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TABLE 1

The pH of Baboon Platelet-Rich Plasma Stored at 22 C in High pH CPD Anticoagulant

Length of Storage (Days):		0	1	2	3	4	5	6	7	8	14	15
Baboon #	Date											
3-Day Storage												
125-77	10/26/81	7.36	7.29	7.55	7.36							
29-78	11/16/81	7.40	7.41	7.45	7.40							
7-Day Storage												
44	5/05/81	7.40	7.69	7.65	7.58	----	----	7.66	7.06			
59	5/12/81	7.42	7.61	7.65	7.59	----	----	7.57	7.22			
48	5/18/81	7.36	7.66	7.50	7.54	7.48	----	----	----			
56-80	11/02/81	7.44	7.31	7.39	7.35	7.30	----	----	7.02			
15-Day Storage												
56-80	1/28/81	7.06	7.20	7.20	----	----	6.68	7.08	7.03	7.00	7.40	7.36
67	2/04/81	7.36	7.41	7.26	----	----	6.88	7.01	----	6.92	7.67	----
56-80	3/04/81	7.36	----	6.92	----	----	6.82	7.01	7.18	7.06	7.65	----

TABLE 2

The Effect of 15 Days of Storage of Baboon Platelet-Rich Plasma at 22 C in High pH CPD Anticoagulant on Platelet Concentration

Length of Storage (Days):		0	1	2	3	7	15
Baboon #	Date	Platelet Count X $10^5/mm^3$					
3-Day Storage							
125-77	10/26/81	4.87	4.07	4.15	4.57	----	----
29-78	11/26/81	3.69	4.07	2.60	2.45	----	----
7-Day Storage							
44	5/05/81	1.40	1.40	1.60	1.44	1.60	----
59	5/12/81	1.99	1.51	1.42	1.56	1.26	----
48	5/18/81	3.20	2.73	2.71	2.44	1.90	----
56-80	11/02/81	4.30	4.15	4.17	4.15	3.03	----
15-Day Storage							
56-80	1/28/81	4.12	5.56	5.19	----	3.78	2.18
67	2/04/81	4.35	4.70	4.51	----	3.95	2.05
56-80	3/04/81	3.60	----	5.18	----	3.80	2.00

TABLE 3

The Effect of 15 Days of Storage of Baboon Platelet-Rich Plasma at 22 C in High pH CPD Anticoagulant on Mean Platelet Volume (u^3) in Nonfixed Aliquots and Aliquots Fixed in 1% Glutaraldehyde in PBS

Length of Storage (Days):		0		1		2		3		7		15	
Sample Preparation:		Fixed u^3	Non-Fixed u^3	Fixed u^3	Non-Fixed u^3	Fixed u^3	Non-Fixed u^3	Fixed u^3	Non-Fixed u^3	Fixed u^3	Non-Fixed u^3	Fixed u^3	Non-Fixed u^3
Baboon #	Date												
3-Day Storage													
125-77	10/26/81	6.62	9.34	----	8.60	6.90	6.62	6.53	7.18	----	----	----	----
29-78	11/16/81	6.71	----	6.21	----	6.27	----	5.86	----	----	----	----	----
7-Day Storage													
44	5/05/81	5.65	7.49	----	----	6.78	7.15	6.20	6.92	7.78	6.02	----	----
59	5/12/81	7.23	7.07	6.45	8.68	5.92	----	6.40	----	5.82	6.46	----	----
48	5/18/81	5.87	----	6.12	----	6.80	7.91	----	----	----	----	----	----
56-80	11/02/81	7.27	7.80	6.01	7.07	5.64	7.03	6.47	6.58	8.35	8.04	----	----
15-Day Storage													
56	1/28/81	5.46	5.12	4.26	4.90	4.93	5.12	----	----	4.35	3.90	3.97	----
67	2/04/81	4.58	5.35	----	----	4.21	----	----	----	----	----	3.74	----
56	3/04/81	----	----	----	----	4.52	5.18	----	----	3.64	3.98	3.56	3.68

FIGURE 1

The posttransfusion ^{51}Cr survival of fresh autologous baboon platelets collected in CPD anticoagulant (either pH 5.5 or pH 9.0), and of autologous baboon platelet-rich plasma stored at pH 7.4 undisturbed at 22 $\pm$ 2 C for up to 15 days.

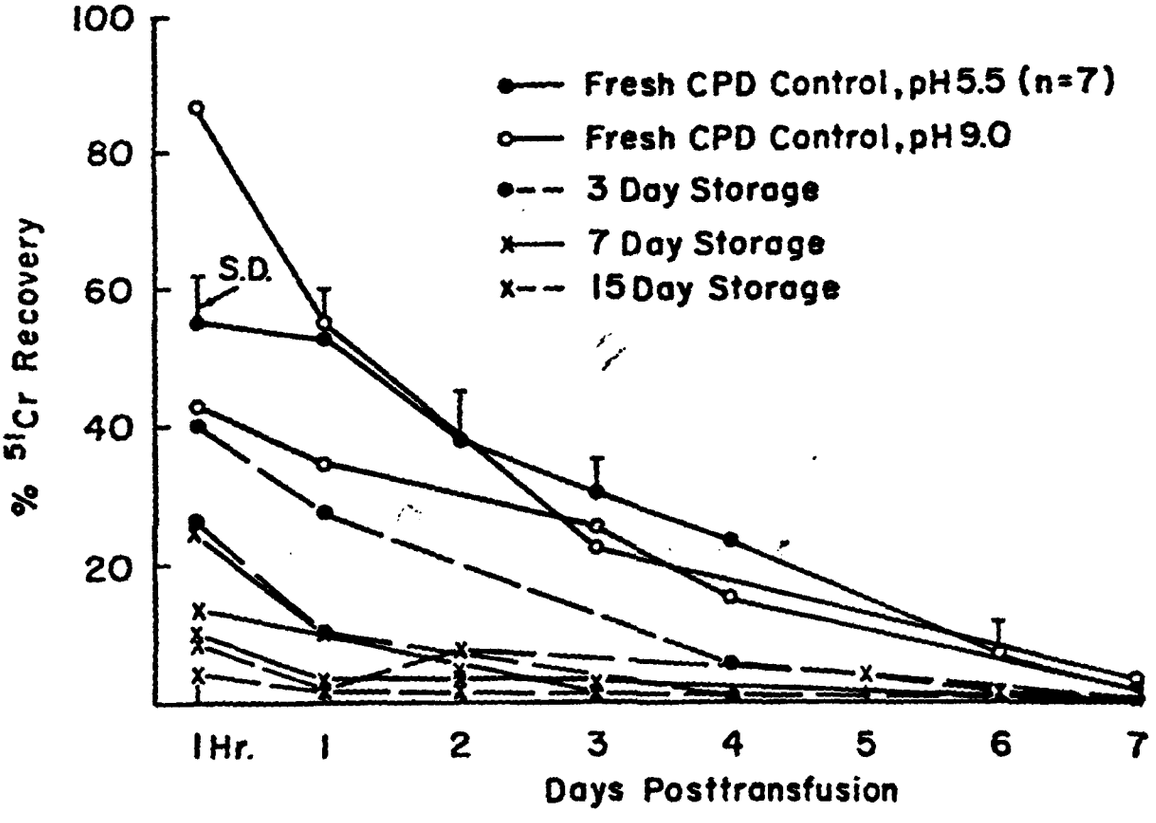

FIGURE 1

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FIGURE 2

The mean posttransfusion ^{51}Cr survival of fresh autologous baboon platelets collected in CPD anticoagulant (either pH 5.5 or pH 9.0) and of autologous baboon platelet-rich plasma stored at pH 7.4 undisturbed at 22 $\pm$ 2 C for up to 15 days.

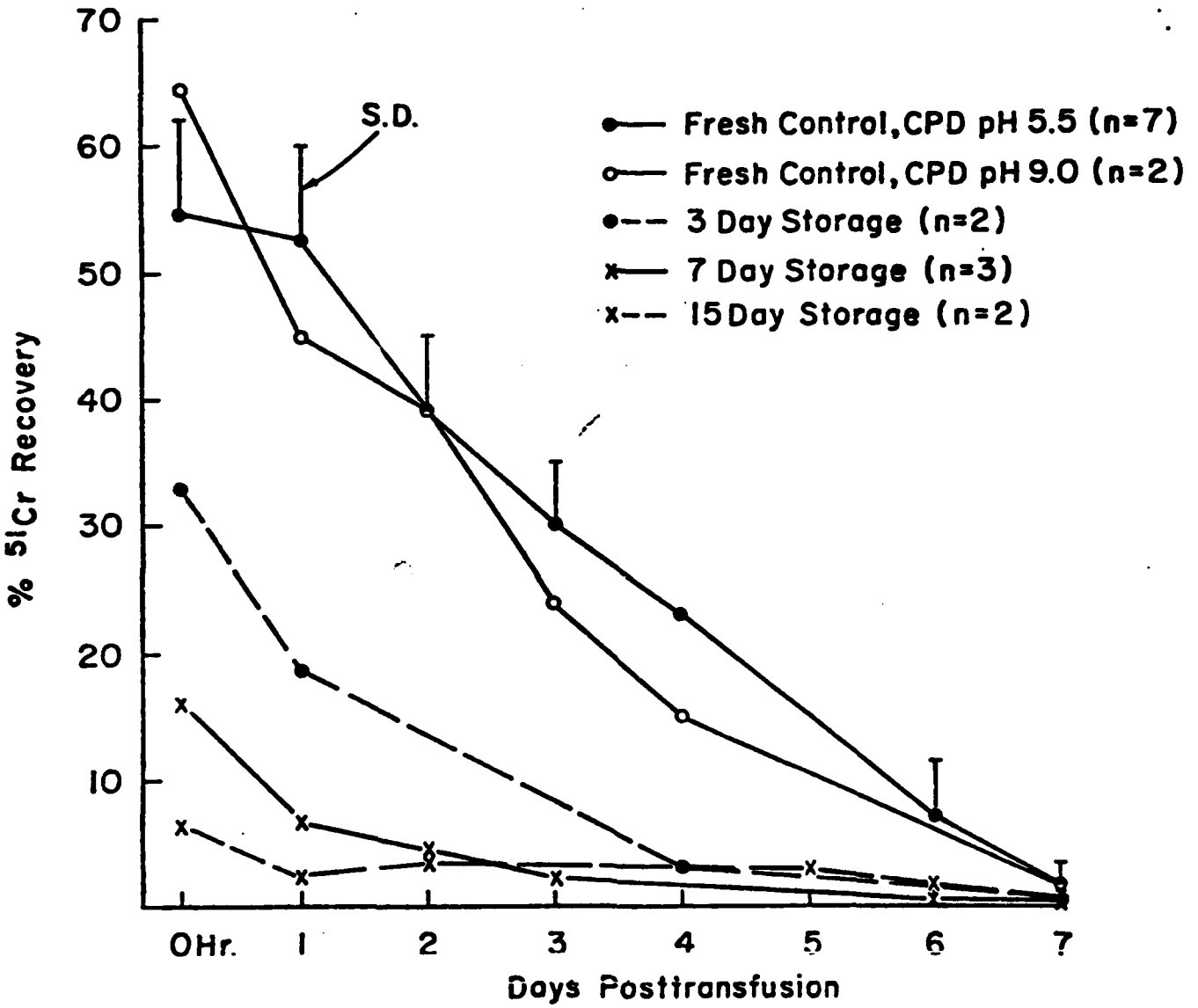

FIGURE 2

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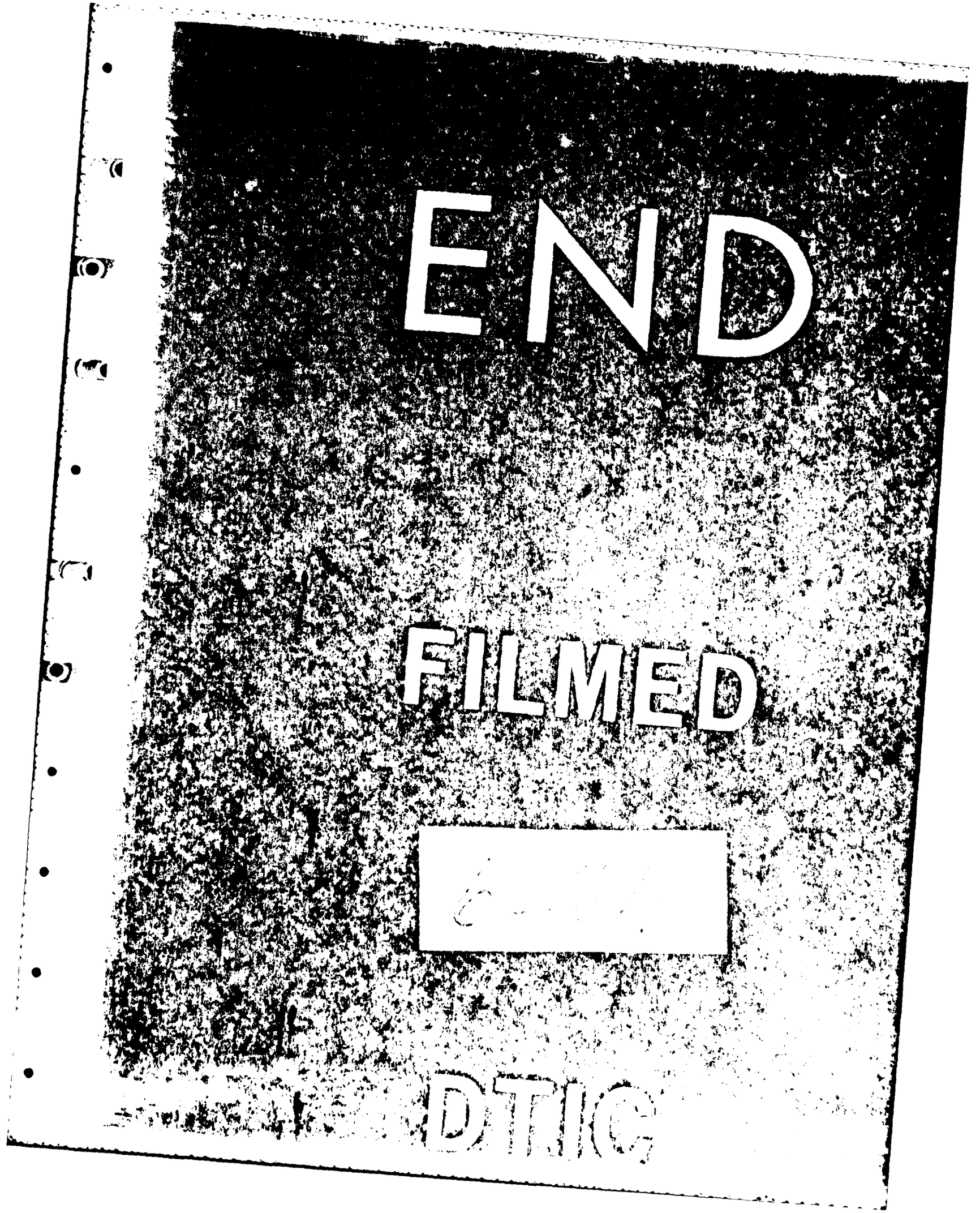
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